No. 0836

Identification of Calcineurin as a Novel Interacting Protein for the Na^+/H^+ Exchanger NHE1 and Its Functional Analysis

Takashi Hisamitsu and Shigeo Wakabayashi

Department of Molecular Physiology,
National Cardiovascular Center Research Institute

Summary

The Na^+/H^+ exchanger NHE1 is activated in response to many kinds of extracellular signals such as hormones, growth factors and mechanical stress, which in turn result in amplification of certain physiological response via change in intracellular pH or Na^+ concentration in the membrane microenvironment. To find the novel binding proteins to NHE1 involved in such regulation, we applied proteomics procedures. NHE1 protein was affinity-purified from PS120 cells stably expressing HA-tagged NHE1 with anti-HA antibodies-conjugated resin. We identified calcineurin (CaN), a Ca^{2+}-dependent Ser/Thr phosphatase, as a new binding partner from the co-purified proteins with HA-tagged NHE1. CaN directly bound to the C-terminus of NHE1. Furthermore, we decided a main CaN binding region located in the NHE1 C-terminus, ^{715}PVITID^{720}, which is highly homologous to PXIXIT sequence known as a CaN-binding motif. Cytoplasmic acidificaton-induced NHE1 activation was not affected in both inhibition of endogenous CaN with FK-506 or cyclosporin and overexpression of constitutively active form of CaN, suggesting that CaN may not extensively modulate NHE1 activity. On the other hand, *in vitro* CaN activity was higher at alkaline pH probably due to increased Ca^{2+} sensitivity in the CaN activator calmodulin, suggesting that CaN activity may be regulated by the change in pH in cells. Now, we hypothesize that the activity of CaN bound to NHE1 molecule is regulated by changes in microenvironmental pH near the NHE1 generated by activated NHE1. To test this hypothesis, we need to determine 1) whether the CaN activity is regulated in NHE1-dependent manner, and 2) how such modulation of CaN activity affects the physiological response.